1 ISOLATION OF DNA

When the DNA examiner has completed his/her analysis, all remaining evidence samples, including extracted DNA which has been dried down using a DNA concentrator/evaporator (Refer to Appendix J, Procedure For Drying Down And Resolubilizing Extracted DNA) or other suitable methods, will be returned to the submitting agency. However, amplified DNA samples WILL NOT be returned to the submitting agency, but instead will be discarded in the designated area to prevent possible transfer of amplified DNA to the remaining evidence samples. If the biological sample deposited on the evidence is consumed during the analysis then the extracted DNA sample and the cutting (in separate tubes) will be returned to the submitting agency with the evidence. Proper case file documentation and chain of custody documentation for these samples must be maintained.

DNA may be extracted from bloodstains, sperm cells, buccal cells, hair, tissue, bone, and other samples. Slightly different extraction procedures are required for each type of specimen and therefore are outlined in this chapter.

It is important to handle all samples aseptically to prevent contamination by extraneous DNA. It is also important to prepare evidence samples at a separate time and/or space from reference samples to prevent possible cross-contamination.

NOTE: For tracking purposes the samples will be listed on the worksheet in the order in which they were processed/handled. The samples will be maintained in the same order throughout the rest of the analysis until the typing gel step. All samples will be processed in accordance with the procedures and policies outlined in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section I, General Documentation and Evidence Handling Requirements, Chapter 3, Contamination Prevention and Detection Procedures, and Section VI, Quality Assurance Program DNA Typing of Biological Materials, Chapter 6, Dedicated PCR Facilities.

Special Precautions:

- Manual DNA extraction and manual PCR setup of evidence samples will be performed at a separate time or space from the manual DNA extraction and manual PCR setup of reference samples. This helps to prevent potential cross-contamination between evidence samples and reference samples.

- Manual DNA extraction from samples containing high levels of DNA (for example, tissue) will be performed after samples expected to contain low levels of DNA (single hairs, small bloodstains, etc.) to minimize the potential for sample-to-sample contamination.

- Disposable gloves will be used at all times. Gloves will be changed frequently to avoid sample-to-sample contamination with DNA and whenever moving between work areas. Gloves will be changed if suspected direct contamination has occurred from the sample DNA.

- To minimize transferring DNA to the disposable gloves a clean Kimwipe will be used to open each microcentrifuge/amplification tube. If the evidence (i.e., stained area or the liquid from the cap of the tube) comes in contact with the disposable glove, change gloves before proceeding to the next stained area, item of evidence, or sample tube.
- Scissors and tweezers will be thoroughly cleaned with a 10% solution of bleach or a solution that will remove/degrade the DNA after cutting each item/stain. Subsequently use isopropanol to remove the

residue left by the chemicals, using special care to remove all residue left on surfaces. A fresh scalpel blade may also be used to cut each item/stain.

- A clean cutting surface will be used for each piece of evidence.
- Disposable plugged pipette tips and microcentrifuge tubes will be used.
- Pipette tips will be changed between samples.
- Reagents will be stored in small quantities to reduce the risk of possible contamination to the stock solution.
- To avoid splashing and minimize aerosols, all liquid will be centrifuged to the bottom of the closed tube before the tube is opened.
- Reagent blank controls will be included with each set of DNA extractions to check for the presence of contaminating DNA in the reagents.
- All work surfaces will be thoroughly cleaned with a 10% bleach solution or a solution that will remove/degrade the DNA. Subsequently use isopropanol to remove the residue left by the chemicals, using special care to remove all residue left on surfaces before setting up the DNA Extraction Work Area. Disposable bench paper will be used to prevent the accumulation of human DNA on permanent work surfaces.
- The quantity of samples handled during a single analysis will be limited to a manageable number. This precaution reduces the risk of sample mix-up and the potential for sample-to-sample contamination.
- A dedicated lab coat will be worn for pre-amplification sample handling when working in the DNA Extraction Work Area.
- A dedicated disposable lab coat will be worn when working with amplified DNA in the PCR Post Amplification Work Area. NOTE: the disposable lab coats are discarded in the post amplification room as necessary.

1.1 TECHNICAL NOTES

1.1.1 Stain extraction buffer lyses the red blood cells (erythrocytes) and helps to remove the DNA from other cellular components.

1.1.2 Sodium dodecyl sulfate (SDS) serves to rupture the white blood cell (leukocyte) nuclear membrane to expose the nucleic acids. It also assists in the denaturation of the nuclear proteins which are attached to the DNA.

1.1.3 Proteinase K (Pro K) is a proteolytic enzyme that reduces proteins to their constituent amino acids. In particular, Pro K removes the histone groups that keep the DNA tightly bound within the cell. The enzymatic activity of Pro K lasts for approximately 2 hours and eventually, it will self-digest.

1.1.4 EDTA is a component of the reagents used in the lysis process which inhibits nuclease activity.

1.1.5 Dithiothreitol (DTT) reduces disulfide bonds that maintain the integrity of the sperm head. Sperm heads do not readily lyse in the absence of heat (56^0C) or DTT.

1.1.6 A differential lysis is included in the procedure for the analysis of mixed stains containing semen and other biological fluid. It includes what is referred to as a sperm and a non-sperm fraction. The non-sperm fraction is contained in the aqueous portion remaining after a gentle lysis treatment of the stain. A more rigorous treatment is conducted for the pelleted material (generally sperm); this is referred to as the sperm fraction.

1.1.7 The DNA IQ™ System is designed to rapidly purify small quantities of DNA, approximately 100 ng or less, and becomes more efficient with samples containing less then 50 ng of DNA.

1.1.8 The DNA IQ™ Lysis buffer contains Guanidine Thiocyanate (GTC), which is a chaotropic agent used to attach the DNA to a silica-coated bead.

1.1.9 The QIAamp® extraction procedure uses spin columns to extract/purify DNA from buccal samples and dried blood stains. The QIAGEN® AL lysis buffer, included in the QIAamp® extraction kit, is a guanidine-based buffer. The guanidine helps to set up the binding conditions needed for the DNA to adhere to the spin column membrane. The QIAGEN® AL lysis buffer also contains a detergent to rupture leukocyte nuclear membranes which exposes the nucleic acids.

1.1.10 The QIAGEN® protease is similar to, but less stringent than, the above mentioned ProK, and serves the same purpose for the breakdown of proteins into their constituent amino acids. The DNA yield reaches a maximum after lysis for 10 min at 56^0 C. Longer incubation times have no effect on the overall yield of the purified DNA.

1.1.11 The QIAGEN® AW1 wash buffer is an ethanol-based stringent wash solution containing a low concentration of guanidine. This wash step removes any non-specific binding to the spin column membrane.

1.1.12 The QIAGEN® AW2 wash buffer is a Tris-based solution containing ethanol which will wash away any salts that are present.

1.1.13 The QIAGEN® AE elution buffer is a Tris-EDTA solution which elutes the DNA attached to the membrane and serves as a stable storage medium.

1.1.14 A random sample will be run with each set of convicted offender and arrestee sample extractions to serve as a verification that the samples are successfully being entered into Combined DNA Index System (CODIS) and the search algorithm is working properly. This sample serves as an internal laboratory control since the DNA profile is not known to the Data Bank analyst and must be verified by the Forensic Biology Section Chief or designee prior to the sizing data being considered acceptable. If a sample must be re-extracted a new random sample must be extracted along with the sample.

1.1.15 Routinely the organic or DNA IQ™ extraction methods are used to isolate DNA from blood/buccal samples obtained in criminal cases and the QIAGEN® BioRobot™ 9604 is used to isolate DNA from convicted offenders blood/buccal or arrestee buccal samples in accordance with the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Manual, Section V - QIAGEN® BioRobot™ 9604 Procedure Manual. However, when necessary convicted offender blood/buccal and arrestee buccal samples may also be extracted using the Organic Extraction Method for Bloodstains and Tissue, the DNA IQ™ Extraction Method For Buccal Cell Type Samples and Bloodstains, the QIAamp® Extraction Procedure, or the QIAamp® 96 Well Extraction Procedure.

1.1.16 The organic, QIAGEN®, and DNA IQ™ Extraction methods will isolate DNA from both human and non-human DNA. However, only the higher primate specific DNA will be quantitated and amplified during the subsequent processing steps.

1.2 EQUIPMENT

1.2.1 Heat block or incubator, 37^0 C

1.2.2 Heat block or incubator, 56^0 C

1.2.3 Microcentrifuge

1.2.4 Vortex mixer

1.2.5 Tweezers

1.2.6 Microcentrifuge tube rack

1.2.7 Pipettes - 10 µL, 20 µL, 100 µL, 200 µL, 1000 µL

1.2.8 8 – Channel Pipette – Range 0.5 µL to 10 µL (for QIAamp® extraction procedure)

1.2.9 Refrigerator/Freezer

1.2.10 Scalpel and blades (for DNA extraction from swabs or hair)

1.2.11 Chisel and hammer (for DNA extraction from bone)

1.2.12 Stereo microscope (for DNA extraction from hair)

1.2.13 Biological Safety Hood

1.2.14 Beaker, 50 mL (for DNA extraction from hair)

1.2.15 Mortar and Pestle (for DNA extraction from bone and teeth)

1.2.16 Incubator, 70^0C (for QIAamp® extraction procedure)

1.2.17 Hole punch (for QIAamp® extraction procedure from dried blood stains)

1.2.18 Centrifuge, equipped with a microplate rotor (for QIAamp® extraction procedure)

1.2.19 Plate orientation adapter for centrifuge (for QIAamp® extraction procedure)

1.2.20 Acrylic block

1.2.21 High Speed Electric Drill (bone procedure)

1.2.22 Rotary shaft tool (tooth procedure - maintained in the firearms section)

1.2.23 1/8" or 9/64" drill bit (bone procedure)

1.3 MATERIALS

1.3.1 Microcentrifuge tubes, 1.5 mL

1.3.2 Transfer pipettes

1.3.3 Sterile ART tips for pipettes, 10, µL, 20 µL, 100 µL, 200 µL, and 1000 µL

1.3.4 Microcentrifuge tube lids

1.3.5 Kimwipes

1.3.6 Conical tubes, 15 mL and 50 mL (for DNA extraction from bone)

1.3.7 Spin-Ease basket (Optional)

1.3.8 White/black paper (for DNA extraction from hair)

1.3.9 Weigh boats (for DNA extraction from bone)

1.3.10 Microscope slides (Optional)

1.3.11 Plastic ziploc bags

1.3.12 Gloves

1.3.13 QAamp® spin columns

1.3.14 QIAamp® 2-mL collection tubes

1.3.15 Parafilm

1.3.16 Round well block (supplied with QIAamp 96 DNA Blood Kit)

1.3.17 8-strip caps for use with the round well block (supplied with QIAamp 96 DNA Blood Kit)

1.3.18 Microplate adhesive tape (supplied with QIAamp 96 DNA Blood Kit)

1.3.19 Airpore tape (supplied with QIAamp 96 DNA Blood Kit)

1.3.20 QIAamp 96 plate (supplied with QIAamp 96 DNA Blood Kit)

1.3.21 S-block (supplied with QIAamp 96 DNA Blood Kit)

1.3.22 13/16" diamond mini cutting disc ("Diamond Life" brand – tooth procedure)

1.3.23 Collection tube rack (supplied with QIAamp 96 DNA Blood Kit)

1.4 REAGENTS

1.4.1 Stain extraction buffer

1.4.2 Proteinase K - 20 mg/mL (Keep on ice.)

1.4.3 TNE

1.4.4 20% Sarkosyl

1.4.5 0.39*M* Dithiothreitol (DTT)

1.4.6 Sterile Type I Water

1.4.7 PCR Digestion Buffer

1.4.8 10% Household bleach (for DNA extraction from bone)

1.4.9 70% Ethanol (for DNA extraction from bone)

1.4.10 Isopropyl Alcohol

1.4.11 95% Ethanol/Reagent Grade (for DNA extraction from bone and use with the QIAamp® blood extraction procedure)

1.4.12 Liquid Nitrogen (for DNA extraction from bone and teeth)

1.4.13 1X phosphate-buffered saline (PBS), pH 7.2 (for DNA extraction from blood/buccal samples)

1.4.14 QIAGEN® protease (for DNA extraction from blood/buccal samples)

1.4.15 QIAGEN® AL lysis buffer (for DNA extraction from blood/buccal samples)

1.4.16 QIAGEN® AW1 wash buffer (for DNA extraction from blood/buccal samples)

1.4.17 QIAGEN® AW2 wash buffer (for DNA extraction from blood/buccal samples)

1.4.18 QAGEN® AE elution buffer (for DNA extraction from blood/buccal samples)

1.4.19 DNA IQ™ Lysis Buffer

1.5 ORGANIC EXTRACTION METHOD FOR BLOODSTAINS AND TISSUE SAMPLES

1.5.1 Cut an approximate 3 mm^2 bloodstain/buccal sample and place into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. If a stain is smeared over a large area of the fabric, more than one microcentrifuge tube may be needed to extract the stain. If the evidential sample consists of tissue, cut a very thin slice of tissue (i.e. 1 mm thick slice of muscle) and place the sample into a 1.5 mL labeled microcentrifuge tube. Alternatively, cellular material may be collected from the tissue by swabbing the sample and placing a portion of the swab into a labeled 1.5 mL microcentrifuge tube. If the evidential sample is a blood flake place sample directly into a 1.5 mL labeled microcentrifuge tube.

NOTE: Process a reagent blank along with each set of samples.

1.5.2 Add 400 µL of stain extraction buffer and 10 µL of Proteinase K to saturate the cutting. If a larger cutting is used, add proportional amounts of stain extraction buffer and Proteinase K until the cutting is saturated.

1.5.3 Mix by hand or light vortexing and pulse spin to force the cutting into the liquid.

1.5.4 Place the tube into a 56^0 C incubator or heat block for a minimum of 2 hours.

NOTE: If the sample has been preserved in Formaldehyde or Formalin add a second 10 µL aliquot of Proteinase K after the 2 hour incubation, then place the tube into a 56^0 C incubator or heat block for an additional 2 hours.

1.5.5 Pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube. If the cutting is small in size and contains a concentrated blood stain, the cutting may be left in the tube.

1.5.6 Remove the lid containing the cutting and discard. (If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence). Place a new lid on the tube containing the liquid.

1.5.7 Proceed to Section 2.5 Microcon® Purification Procedure

1.6 ORGANIC/ DNA IQ™ EXTRACTION METHOD FOR MIXED BODY FLUID STAINS (DIFFERENTIAL PROCEDURE)

OPTION: Mixed body fluid samples may also be extracted using the DNA IQ™ extraction method for mixed body fluid stains outlined in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section IV - BioMek®2000 Automation Workstation Procedures Manual.

1.6.1 Cut an approximate 3 mm^2 stain or a portion of a cotton swab proportional to the number of sperm identified. Place the stain into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. If the stain is smeared over a large area on the fabric or the body fluids on a swab are weak and more than one swab must be used, more than one microcentrifuge tube may be needed to extract the stain.

1.6.1.1 If a sample contains a weak smear over a large surface area the sample should be placed in several 1.5 mL microcentrifuge tubes and the entire sample condensed into one sample tube during the purification step, Section 2.5 Microcon® Purification Procedure or Section 2.6 DNA IQ™ Purification Procedure.

NOTE: Process a reagent blank along with each set of samples.

1.6.2 Add:

400 µL TNE
25 µL 20% Sarkosyl
75 µL Sterile Type I Water
5 µL Proteinase K

in proportional amounts to saturate the cutting.

1.6.3 Mix by hand or light vortexing then pulse spin to force the cutting into the liquid.

1.6.4 Place the tube into a 37^0C incubator or heat block for a minimum of 2 hours.

1.6.5 Pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube.

1.6.6 Using a pipette, carefully transfer all but approximately 50 µL of the supernatant into a new 1.5 mL labeled tube with a lid. Be careful not to dislodge or disturb the pellet on the bottom of the tube. The supernatant removed from the pellet is the NON-SPERM FRACTION.

1.6.7 At this stage set the non-sperm fraction tube aside and wait until the sperm fraction is ready, then proceed to Section 2.5, Microcon® Purification Procedure or Section 2.6, DNA IQ™ Purification Procedure.

NOTE: If necessary the sample(s) may be capped and left at room temperature overnight before proceeding.

1.6.8 Remove and discard the old lid and cutting from the original tube containing the pellet. (If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence). Place a new colored lid on the tube. This tube contains the SPERM FRACTION.

1.6.9 Wash the pellet as follows: Resuspend the pellet in 500 µL of PCR digestion buffer by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Using a pipette with a sterile 1 mL pipette tip, remove all but 50 µL of the supernatant and discard.

1.6.10 Repeat the wash in step 1.6.9 an additional 2 times. If a low sperm count has been determined, the sperm pellet may be washed up to 5 times. If the SPERM FRACTION will be purified using the DNA IQ™ isolation method, proceed to Section 2.6, DNA IQ™ Purification Procedure with both the sperm and non-sperm fractions, otherwise proceed to step 1.6.11.

NOTE: If necessary the sample(s) once capped can remain at room temperature overnight before proceeding.

OPTION: If a sperm search has not previously been conducted, remove 3 µL of the supernatant and spot the sample onto a glass microscope slide for examination.

1.6.11 Add to each sperm fraction or reagent blank tube:

150 µL TNE
50 µL 20% Sarkosyl
40 µL 0.39*M* DTT
150 µL Sterile Type I Water
10 µL Proteinase K

1.6.12 Mix by hand or light vortexing then place the tube into a 56^0 C incubator or heat block for 2-3 hours.

1.6.13 Pulse spin the tube.

1.6.14 Proceed to Section 2.5, Microcon® Purification Procedure, with both the sperm and non-sperm fractions.

1.7 ORGANIC/ DNA IQ™ EXTRACTION METHOD FOR ISOLATING DNA FROM HEAT FIXED AND PERMOUNTED SLIDES/SMEARS (DIFFERENTIAL PROCEDURE)

NOTE: If the slide/smear has not been heat fix and permounted, skip to step 1.7.5.

1.7.1 Place the stained, fixed and permounted smear/slide into a clean glass Petri dish with a cover.

1.7.2 Pour a sufficient volume of xylene over the smear/slide until completely submerged.

1.7.3 Incubate the smear/slide overnight at room temperature. If the coverslip does not float off easily, continue to soak the smear/slide in xylene until the coverslip can be removed easily. NOTE: smears/slides that have been mounted with an excess amount of permount may need to be incubated in the xylene solution of as much as 5 days.

1.7.4 Once the coverslip floats off, remove the smear/slide from the Petri dish and air dry for a minimum of 5 minutes. DO NOT DISCARD THE COVERSLIP.

1.7.5 Scrape the cellular material off the smear/slide with a clean, unused scalpel or razor blade and place it into a labeled 1.5 mL microcentrifuge tube.

1.7.6 Remove half the cotton from a sterile cotton tipped swab. Wet the remaining portion of the swab attached to the stick slightly with sterile water and then use the stick and swab to scrape smear/slide to remove any remaining cellular material.

1.7.7 Remove the cotton from the stick and place it into the 1.5 mL microcentrifuge tube with the respective scrapings from the smear/slide or break off the portion of the stick with the swab attached and place into the 1.5 mL tube.

1.7.8 Retain the smear/slide along with the coverslip until it is certain that DNA has successfully been obtained. Cells can stick to both the slide and the coverslip.

NOTE: Process a reagent blank along with each set of samples

1.7.9 To the 1.5 mL tube add:

400 µL TNE
25 µL 20% Sarkosyl
75 µL Sterile Type I Water
5 µL Proteinase K

in proportional amounts to saturate the cutting.

1.7.10 Mix by hand or light vortexing then pulse spin to force the cutting into the liquid.

1.7.11 Place the tube into a 37^0C incubator or heat block for a minimum of 2 hours.

1.7.12 Pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000

rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube.

1.7.13 Using a pipette, carefully transfer all but approximately 50 µL of the supernatant into a new 1.5 mL labeled tube with a lid. Be careful not to dislodge or disturb the pellet on the bottom of the tube. The supernatant removed from the pellet is the NON-SPERM FRACTION.

1.7.14 At this stage set the non-sperm fraction tube aside and wait until the sperm fraction is ready, then proceed to Section 2.5, Microcon® Purification Procedure or Section 2.6, DNA IQ™ Purification Procedure.

NOTE: If necessary the sample(s) may be capped and left at room temperature overnight before proceeding.

1.7.15 Remove and discard the old lid and cutting from the original tube containing the pellet. (If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence). Place a new colored lid on the tube. This tube contains the SPERM FRACTION.

1.7.16 Wash the pellet as follows: Resuspend the pellet in 500 µL of PCR digestion buffer by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Using a pipette with a sterile 1 mL pipette tip, remove all but 50 µL of the supernatant and discard.

1.7.17 Repeat the wash in step 1.7.16 an additional 2 times. If a low sperm count has been determined, the sperm pellet may be washed up to 5 times. If the SPERM FRACTION will be purified using the DNA IQ™ isolation method, proceed to Section 2.6, DNA IQ™ Purification Procedure with both the sperm and non-sperm fractions, otherwise proceed to step 1.7.18.

NOTE: If necessary the sample(s) once capped can remain at room temperature overnight before proceeding.

OPTION: If a sperm search has not previously been conducted, remove 3 µL of the supernatant and spot the sample onto a glass microscope slide for examination.

1.7.18 Add to each sperm fraction or reagent blank tube:

150 µL TNE
50 µL 20% Sarkosyl
40 µL 0.39*M* DTT
150 µL Sterile Type I Water
10 µL Proteinase K

1.7.19 Mix by hand or light vortexing then place the tube into a 56^0 C incubator or heat block for 2-3 hours

1.7.20 Pulse spin the tube

1.7.21 Proceed to Section 2.5, Microcon® Purification Procedure, with both the sperm and non-sperm fractions.

References:

1. Dimo-Simonin, N., Grange, F., Brandt-Casadevall, C., PCR-based forensic testing of DNA from stained cytological smears. J. Forensic Sci. 1997:42(3):506-509.

2. Gall, K., Pavicic, D., Pavelic, J., Audy-Jurkovic, S., Pavelic, K., PCR amplification of DNA from stained cytological smears. J. Clin. Pathol. 1993;46:378-379.

3. Pavelic, J., Gall-Troselj, K., Bosnar, M.H., Kardum, M.M., and Pavelic, K., PCR amplification of DNA from archival specimens, a methological approach. Neoplasma, 1996;43(2):75-81.

4. Sweet, D., Hildebrand, D., Phillips, D., Identification of a skeleton using DNA from teeth and a PAP smear. J. Forensic Sci. 1999;44(3):630-633.

5. Poljak, M., Barlic, J., Rapid, simple method of extracting DNA from archival Papanicolaou-stained cervical smears. Acta Cytol. 1996;40:374-375.

6. Part 1: Protocol For Recovery of DNA From Biological Evidence Material, Section C: Collection of Cellular Material From Microscope Slides, Oakland Police Department Criminalistics Laboratory DNA Methods Manual, Page 1-2, June 20, 2005.

1.8 ORGANIC EXTRACTION METHOD FOR BONE

1.8.1 Using a Kimwipe and 95% ethanol vigorously clean the outer surface of the bone sample. Repeat this step 2 –3 more time to remove any surface dirt or contaminates.

1.8.2 Place bone into a weigh boat or lay on a piece of clean butcher paper inside of a biological safety hood to dry.

1.8.3 The bone sample (i.e., small in size) may be pulverized using liquid nitrogen (1.8.3.1A) or an electric drill and bit (1.8.3.2A) following the procedures listed below:

1.8.3.1A Pulverizing the bone sample using liquid nitrogen: Place the bone fragment(s) into a clean mortar then pour a small volume of liquid nitrogen over the top of the bone. Ensure there is a sufficient amount of liquid nitrogen in the mortar to cover the bone fragment(s).

1.8.3.1B Allow the bone to sit in the liquid nitrogen for 30 to 60 seconds, then pour off the excess liquid nitrogen into a separate container. Using a pestle crush the bone fragments. Place a piece of parafilm over the top of the mortar and pestle to prevent the pulverized bone fragments from flying out of the mortar.

1.8.3.1C Transfer a small portion of the pulverized bone to a 1.5 mL microcentrofuge tube. Proceed to step 1.8.4.

1.8.3.2A Pulverizing the bone sample using a drill and bit(s): Initially clean a 1/8” or 9/64” drill bit with 10% bleach and a Kimwipe followed by 95% ethanol.

1.8.3.2B Place the bone sample in a hood on a clean sheet of butcher paper.

1.8.3.2C While holding the bone sample firmly use an electric drill and either the cleaned 1/8” or 9/64” drill bit and drill a hole approximately 1.0 mm deep.

1.8.3.2D Tap the bone gently on the butcher paper to dislodge the surface bone powder. Discard the sheet of butcher paper and replace with a new sheet of butcher paper.

1.8.3.2E Clean the 1/8” or 9/64” drill bit with a Kimwipe and 95% ethanol.

1.8.3.2F Place the drill bit back into the hole in the bone and drill approximately 3-5 mm further into the bone.

1.8.3.2G Tap the bone gently on the butcher paper to dislodge the bone powder onto the butcher paper.

1.8.3.2H Transfer a sample of bone powder at minimum the size of a PEA to a clean 1.5 mL microcentrifuge tube. Proceed to step 1.8.4.

1.8.4 Add 1 mL of stain extraction buffer and 35 µL of Proteinase K to the pulverized/powder bone sample in proportional amounts to saturate the sample.

NOTE: Process a reagent blank along with each set of samples.

1.8.5 Place the tube into 56° C heat block or incubator. After one hour of incubation, mix thoroughly and re-secure the cap of the tube. Continue to incubate the bone sample in the 56° C heat block overnight.

1.8.6 Proceed to Section 2.5, Microcon® Purification Procedure

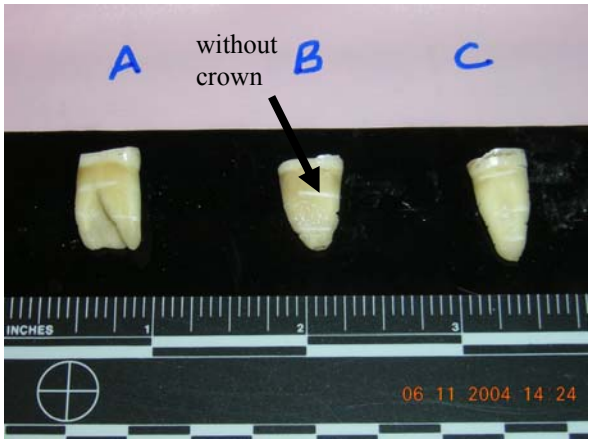
1.9 ORGANIC EXTRACTION METHOD FOR TEETH[1]

NOTE: Molars are the tooth of choice for DNA recovery.

1.9.1 Clean the outer surface of the tooth with a Kimwipe and 10% bleach (avoid introducing the bleach solution into any cracks in the tooth). Subsequently removed the 10% bleach residue using a Kimwipe and sterile water followed by isopropanol.

1.9.2 While working in a hood, using a sterile diamond saw blade and a rotary shaft tool cleaned with bleach and isopropanol, cut/grind away the upper crown portion of the tooth until the pulp chamber becomes visible. In addition, make small nicks in the sides of the tooth to facilitate crushing the lower portion of the tooth. Refer to the diagrams below.

NOTE: It will take between 5 and 10 minutes to remove the crown of the tooth.



1.9.3 To gain access to the pulp, place the tooth minus the crown into a small sterile ziploc plastic bag. Insert the ziploc plastic bag into a second ziploc bag. Ensure the ziploc bags are sealed.

1.9.4 Lay the ziploc plastic bag on a hard surface. Using a hammer pulverize the tooth sample inside of the ziploc plastic bag. Be careful not to puncture the ziploc plastic bag.

1.9.5 While holding the plastic bag with the ziploc portion of the plastic bag up, shake the sample to transfer the pulverized sample to the corner of the ziploc plastic bag. Open the ziploc plastic bags and transfer the pulverized/crushed tooth to a 1.5 mL microcentrifuge tube.

NOTE: Process ~~a random sample and~~ a reagent blank along with each set of samples.

1.9.6 Add 400 µL of stain extraction buffer and 10 µL of Proteinase K proportionally to the pulverized tooth sample.

1.9.7 Mix by hand or light vortexing and pulse spin to force the pulverized tooth sample into the liquid.

1.9.8 Place the tube into a 56°C incubator or heat block for a minimum of 2 hours.

1.9.9 Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to force the pulverized tooth sample to the bottom of the tube.

1.9.10 Proceed to Section 2.5, Microcon® Purification Procedure

Reference:

1 Gaytmenn, et.al. “Quantification of Forensic DNA from Various Regions of Human Teeth, JFS, Vol.48, No. 3, pp.622-625.

1.10 DNA IQ™ EXTRACTION METHOD FOR BUCCAL CELL TYPE SAMPLES AND BLOODSTAINS

OPTION: Non-differential extractions may also be conducted using the DNA IQ™ extraction procedure outlined in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section IV - BioMek®2000 Automation Workstation Procedures Manual.

The portion size of the swab or bloodstain removed for DNA extraction should be judged based on a number of criteria, such as whether the stain appears dilute (for bloodstains) or if the sample may be heavily soiled or possibly degraded. Examine the bloodstain or buccal swab and remove a reasonable portion for DNA extraction. For example, only a 3 mm^2 section may be all that is necessary to remove from a heavily bloodstained item.

NOTE: Process a reagent blank along with each set of samples.

1.10.1 Cut an approximate 3 mm^2 blood stain or other biological stain and place into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. If a stain is smeared over a large area of fabric, more than one microcentrifuge tube may be needed to extract the stain. If the sample is a buccal swab, remove a small portion of the swab and place into a labeled microcentrifuge tube.

NOTE: Prior to the addition of the DNA IQ™ Lysis buffer, DTT at the concentration described in the reagent preparation section (Appendix B) MUST be added.

1.10.2 Add 250 uL of DNA IQ™ Lysis buffer. If the biological sample is dispersed over a large area of the substrate, add up to 325 uL of DNA IQ™ Lysis buffer to the microcentrifuge tube.

1.10.3 Vortex vigorously for 20-30 seconds, then pulse spin to force the cutting into liquid.

1.10.4 Place the microcentrifuge tube into a 56ºC heat block for a minimum of 30 minutes. If the sample is deposited on FTA paper, place the microcentrifuge tube into a 95° C heat block for a minimum of 30 minutes.

1.10.5 Vortex vigorously for 20-30 seconds, then pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. Alternatively, a Spin-Ease basket may be used instead of placing the cutting in the lid of the tube. If the entire biological sample deposited on the evidence was consumed the cutting will be returned with the evidence.

NOTE: Samples can be left at room temperature in DNA IQ™ Lysis buffer for up to 24 hours after heating and centrifugation before proceeding to the BioMek® 2000 Automation Workstation. If the samples are stored in a refrigerator before proceeding to Section 2, DNA Purification, place the samples in a 56ºC heat block for 5 minutes to resolubilize the samples.

1.10.6 Proceed to Section 2.6, DNA IQ™ Purification Procedure

1.11 DNA IQ™ EXTRACTION METHOD FOR HAIR, HIGHLY CONCENTRATED BLOODSTAINS, AND LOW LEVEL SAMPLES

NOTE: This procedure may also be used for samples that are believed to have a low concentration of DNA (i.e., envelopes, stamps, cigarette butts) or highly concentrated bloodstain (i.e., dried blood flakes).

1.11.1 Follow the steps outlined below for the extraction of hairs. Proceed to step 1.11.2 for all other sample types:

1.11.1.1 Using clean tweezers, place the hair on a clean piece of white or black paper, whichever is appropriate, and examine under a stereo microscope for the presence of sheath material. Note the presence of any body fluids on the hair.

1.11.1.2 Wash the hair to reduce surface dirt and contaminants by immersing the hair in sterile Type I Water in a clean 50 mL beaker. If the hair contains a biological fluid that is important to the investigation DO NOT wash the hair.

1.11.1.3 Return the hair to the stereo microscope. Use a clean scalpel blade to cut a 0.5 to 1 cm portion from the root end of the hair and then place the hair root into a 1.5 mL microcentrifuge tube and proceed to step 1.11.3.

NOTE: Process a reagent blank along with each set of samples.

1.11.2 Cut an approximate 3 mm^2 blood stain or other biological stain and place into a labeled 1.5 mL microcentrifuge tube with a depression in the lid

1.11.3 Add to the 1.5 mL microcentrifuge tube:

37.5 µL TNE
12.5 µL 20% Sarkosyl
10.0 µL 0.39*M* DTT
32.5 µL Sterile Type I Water
10.0 µL Proteinase K

in proportional amounts to saturate the cutting.

1.11.4 Mix by hand or lightly vortex, then pulse spin the microcentrifuge tube to force the sample into the liquid.

1.11.5 Place the tube into a 56ºC incubator or heat block for minimum of 1 hour.

1.11.6 Pulse spin the tube in a microcentrifuge for 10 seconds to force the condensate to the bottom of the tube.

1.11.7 Proceed to Section 2.6, DNA IQ™ Purification Procedure

1.12 DNA IQ™ EXTRACTION METHOD FOR TISSUE SAMPLES

1.12.1 Cut a very thin slice of tissue (i.e. 1 mm thick slice of muscle) and place the sample into a labeled 1.5 mL microcentrifuge tube. If the tissue sample is degraded a slightly larger portion of the sample may be used. Alternatively, cellular material may be collected from the tissue by swabbing the sample and placing a portion of the swab into a labeled 1.5 mL microcentrifuge tube.

NOTE: Process a reagent blank along with each set of samples.

1.12.2 Add 90 µL of 1X $CaCl_2$ buffer and 10 µL of Proteinase K to saturate the sample.

1.12.3 Mix by hand or light vortexing and pulse spin to force the sample into the liquid.

1.12.4 Place the tube into a 56ºC incubator or heat block for a minimum of 2 hours.

NOTE: If the sample has been preserved in Formaldehyde or Formalin add a second 10 µL aliquot of Proteinase K after the 2 hour incubation, then place the tube into a 56° C incubator or heat block for an additional 2 hours.

1.12.5 Spin the 1.5 mL microcentrifuge tube at ~12,000 rpm for 5 minutes to pellet any undigested debris.

Proceed to Section 2.6, DNA IQ™ Purification Procedure

1.13 QIAamp® EXTRACTION PROCEDURE – ARRESTEE AND OFFENDER SAMPLES

NOTE: Process a reagent blank and random sample along with each set of samples.

1.13.1 Dried blood stains: Cut a 3 - 5 mm^2 blood stain or using a hole puncher, punch one hole from the dried blood stain and transfer to a labeled 1.5 mL microcentrifuge tube using tweezers. If a hole punch is used, the hole punch should be cleaned between each punch using a Kimwipe and isopropanol. Add 180 µL PBS buffer to each tube and then pulse spin to force the bloodstain into the buffer. Incubate at 56°C for one hour, then proceed to step 1.13.2.

1.13.2 Add 20 µL of the QIAGEN® protease to each tube and vortex for 15 seconds to thoroughly mix the sample.

WARNING!

The QIAGEN® AL lysis contains Guanidine Hydrochloride. This chemical will produce chlorine gas when mixed with bleach. When cleaning the counter tops after using the QIAGEN® extraction method wash the counter top first with water, then isopropanol, followed by a 10% bleach solution to disinfect the area.

1.13.3 Mix the QIAGEN® AL lysis buffer by shaking thoroughly before use. If a precipitate is observed, incubate at 70°C until dissolved. Add 200 µL of the QIAGEN® AL lysis buffer to each tube. Vortex the tube for 15 seconds to thoroughly mix the sample. **The QIAGEN® AL lysis buffer is light sensitive and should not be left in the light longer than is necessary**.

1.13.4 Incubate the tube in a 56°C heat block for 10 minutes.

1.13.5 Place the tube into a microcentrifuge and pulse spin the sample to remove condensation from lid.

1.13.6 Add 200 µL 95% ethanol to each tube. Vortex the tube for 15 seconds to thoroughly mix the sample.

1.13.7 Place the tube into a microcentrifuge and pulse spin the sample to remove condensation from lid.

1.13.8 Transfer the supernatant from the tube to a labeled QIAamp® spin column that is inside of a 2.0 mL collection tube. Centrifuge the sample for 1 minute at 8,000 rpm. Be careful not to apply the sample to the rim of the spin column. Sample that has been deposited on the rim during the centrifugation will be transferred from the tube to the inside of the microcentrifuge.

1.13.9 Discard the 2.0 mL collection tube containing the filtrate and place the QIAamp® spin column into a clean 2.0 mL collection tube.

1.13.10 Add 500 µL of the QIAGEN® AW1 wash buffer and centrifuge for 1 minute at 8,000 rpm.

1.13.11 Discard the 2.0 mL collection tube containing the filtrate and place the QIAamp® spin column into a clean 2.0 mL collection tube.

1.13.12 Add 500 µL of the QIAGEN® AW2 wash buffer and centrifuge for 3.5 minutes at 13,200 rpm.

1.13.13 Discard the 2.0 mL collection tube containing the filtrate and place the QIAamp® spin column into a clean, labeled 1.5 mL microcentrifuge tube.

NOTE: Use capless tubes or remove the caps from the tubes before centrifugation.

1.13.14 Add 50 µL of the QIAGEN® AE Elution Buffer or Type 1 water to each QIAamp® spin column.

1.13.15 Incubate the sample at room temperature for 1 minute.

1.13.16 Place the 1.5 mL microcentrifuge tube containing the QIAamp® spin column into a microcentrifuge and spin for 1 minute at 8,000 rpm.

1.13.17 Discard the QIAamp® spin column and cap the 1.5 mL microcentrifuge tube containing the extracted DNA sample.

1.13.18 Proceed to Section 5, PCR Amplification.

1.14 QIAamp® 96 WELL EXTRACTION PROCEDURE – ARRESTEE AND OFFENDER SAMPLES

NOTE: Process a reagent blank and random sample along with each set of samples.

1.14.1 **Dried blood stains and/or buccal samples:** Using a 6 mm^2 hole puncher, punch one hole from the dried blood stain onto a Kimwipe. Using tweezers carefully fold the punch in half and then transfer to the appropriate position in the 96 well round well block. **If a hole punch is used, the hole punch and tweezers should be cleaned between each punch using a Kimwipe and isopropanol**. Add 195 µL PBS buffer to each of the wells, then cap the wells with an 8-strip cap. Pulse spin the 96 well round well block to force the punch into the buffer. Incubate at 70°C in an oven for one hour. Place an acrylic block on top of the plate to prevent the caps from popping off. Once incubation is complete, pulse spin the 96 well round well block to remove condensation from the caps, then proceed to step 1.14.2.

NOTE: When the procedure makes reference to pulse spinning the sample(s), allow the centrifuge to reach approximately 3,000 rpm and then turn off the centrifuge and allow the rotor to come to rest.

1.14.2 Add 20 µL of the QIAGEN® protease to each well. Cap all wells and shake the 96 well round well block using two hands to thoroughly mix the samples. Pulse spin the 96 well round well block to remove condensation from the caps.

WARNING!

The QIAGEN® AL lysis buffer contains Guanidine Hydrochloride. This chemical will produce chlorine gas when mixed with bleach. When cleaning the counter tops after using the QIAGEN® extraction method wash the counter top first with water, then isopropanol, followed by a 10% bleach solution to disinfect the area.

1.14.3 Mix the QIAGEN® AL lysis buffer by shaking thoroughly before use. If a precipitate is observed, incubate at 70°C until dissolved. Add 200 µL of the QIAGEN® AL lysis buffer to each well. Cap each well, then using two hands shake the 96 well round well block for 15 seconds to thoroughly mix the samples. **The QIAGEN® AL lysis buffer is light sensitive and should not be left in the light longer than is necessary.**

1.14.4 Incubate the 96 well round well block in a 70°C incubator for 10 minutes. Place an acrylic block on top of the round well block to prevent the caps from opening.

1.14.5 Place the 96 well round well block into a centrifuge with a plate rotor and pulse spin the samples to remove condensation from lid.

1.14.6 Add 200 µL 95% ethanol to each well. Seal the wells using new caps for the round well blocks. Shake using two hands the 96 well round well block for 15 seconds to thoroughly mix the samples.

1.14.7 Place the 96 well round well block into a centrifuge with a plate rotor and pulse spin the samples to remove condensation from cap.

1.14.8 Place a QIAamp 96 well plate on top of an S-block. Mark the plate on the side for identification.

1.14.9 Transfer the supernatant from the round well block to the corresponding well of the QIAamp® 96 well plate. Be careful not to apply the sample to the rim of QIAamp® 96 well plate. Sample that has been deposited on the rim may cross contaminate other samples.

1.14.10 Seal the QIAamp 96 well plate with an airpore tape sheet. Load the S-block and QIAamp 96 well plate into the centrifuge carrier, then place the carrier into the rotor basket. Centrifuge the samples for 4 minutes at approximately 6,000 rpm.

1.14.11 Remove the airpore tape sheet and add 500 µL of the QIAGEN® AW1 wash buffer to each well.

1.14.12 Seal the QIAamp 96 well plate with an airpore tape sheet. Load the S-block and the QIAamp 96 well plate into the centrifuge carrier, then place the carrier into the rotor basket. Centrifuge the samples for 2 minutes at approximately 6,000 rpm.

1.14.13 Remove the airpore tape sheet and add 500 µL of the QIAGEN® AW2 wash buffer to each well.

1.14.14 Seal the QIAamp 96 well plate with an airpore tape sheet. Load the S-block and the QIAamp 96 well plate into the centrifuge carrier, then place the carrier into the rotor basket. Centrifuge the samples for 15 minutes at approximately 6,000 rpm.

NOTE: The heat generated during centrifugation allows for evaporation of any residual ethanol on the membrane.

1.14.15 Place the white orientation adapter ring on top of a rack of collection tubes, then place the QIAamp 96 well plate on top of the adapter. Ensure the pieces fit snugly together. **IF THE PIECES DO NOT FIT SNUGLY CHECK THE ORIENTATION OF THE ADAPTER**.

1.14.16 To elute the DNA, add 50 µL of the QIAGEN® AE Buffer or Type 1 water to each well. Seal the QIAamp 96 well plate with an airpore tape sheet and incubate for 1 minute at room temperature.

1.14.17 Load the plate combination into the centrifuge carrier, then place the carrier into the rotor bucket. Centrifuge the samples for 4 minutes at approximately 6,000 rpm.

NOTE: If a partial plate was processed, seal the used wells with adhesive tape and label “used”.

1.14.18 Seal the wells of the collection tubes using the 8-strip caps. Label tubes appropriately.

1.14.19 Proceed to Section 5, PCR Amplification.

♦**END**